Amendments to the Specification:

Please replace the three paragraphs at page 2, lines 3-21 with the following amended paragraphs:

Development of ~~Onconase~~ ONCONASE® conjugates for human therapeutics has been slow. ~~Onconase~~ ONCONASE® is derived from amphibian tissue and trace contaminants present in the purified preparation stimulate undesirable immune responses in humans. This side-effect has led to production of a recombinant form of the protein (Newton, *et al., Protein Engineering* 10:463-470 (1997) and PCT published application WO 97/38112).

However, expression of active recombinant ~~Onconase~~ ONCONASE® has been problematic. ~~Onconase~~ ONCONASE® requires a pyroglutamic acid at the N-terminus for activity. Unfortunately, ~~Onconase~~ ONCONASE® with a N-terminal glutamine is not expressed by bacteria but accumulates in insoluble inclusion bodies. To increase bacterial expression of soluble ~~Onconase~~ ONCONASE®, methionine has been appended to the N-terminus. However, this modification of the protein prevents the formation of the pyroglutamic acid necessary for activity. Therefore, it has been necessary to engineer ~~Onconase~~ ONCONASE® with an N-terminal methionine only to remove it for activity. The cleaved and the uncleaved proteins must then be separated to obtain a pure composition of high purity and activity.

Other problems have ~~arisin~~ arisen in the manufacture of ~~Onconase~~ ONCONASE®-based fusion proteins. It has been difficult to fuse recombinant ~~Onconase~~ ONCONASE® in frame to ligand binding moieties and retain proper folding of both the ~~Onconase~~ ONCONASE® and the ligand binding moiety. This has limited the use of ~~Onconase~~ ONCONASE® in targeted cell killing to only those compounds that can be chemically conjugated.

Please replace the paragraph at page 2, line 28 bridging to page 3, line 2 with the following amended paragraph:

This invention provides for new recombinant ribonuclease proteins. The proteins, unlike ~~Onconase~~ ONCONASE®, are expressed well by bacteria without an N-terminal

methionine. This is due largely to the presence of a signal peptide that is cleaved by the bacteria. The ribonucleases are then secreted into the bacterial media. The soluble expression of these ribonucleases allows the proteins of this invention to be fused in-frame with ligand binding moieties to form cytotoxic fusion proteins.

Please replace the paragraph at page 13, line 20 bridging to page 14, line 7 with the following amended paragraph:

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to ribonuclease with the amino acid sequence encoded in SEQ ID NO:2 can be selected to obtain only those antibodies that are specifically immunoreactive with ribonuclease and not with other proteins, except for polymorphic variants, alleles, and closely related interspecies homologs of ribonuclease. This selection may be achieved by subtracting out antibodies that cross react with molecules such as ~~Onconase~~ ONCONASE®. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, ANTIBODIES, A LABORATORY MANUAL (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

Please replace the paragraph at page 33, line8, bridging to page 34, line 5 with the following amended paragraph:

The RNAse molecules are uniquely adapted for gene therapy applications. They can be fused to other therapeutic agents, for example, they could be fused to an anti-B cell lymphoma antibody, an anti-transferrin receptor antibody or an anti-colon cancer antibody. As mentioned above, native ~~Onconase~~ ONCONASE® has anti-tumor effects in vivo and preferentially kills rapidly dividing cells stimulated by serum or growth promoting agents such as ras. The RNAses of this invention can be used in a similar manner. The RNAses of this invention are readily internalized in the cell. Their activity can be further facilitated by joining them to a nuclear localization signal (NLS) and the like to redirect the molecules within the cell. Of particular use in tumor cells would be to target telomerase, an enzyme subject to degradation by ribonuclease.

Please replace the paragraph at page 34, lines 23-30 with the following amended paragraph:

Studies with ~~Onconase~~ ONCONASE® have indicated other potential uses. It has been found that ~~Onconase~~ ONCONASE® synergizes with ras in microinjection studies. Onconase® does not synergize with ras when it enters the cell via its own routing but requires a CAAX motif to localize ras at the plasma membrane (C=Cys, A = an aliphatic amino acid, X = S,M,C,A, or Q, an example is Cys-Val-Ile-Met). Importantly this type of sequence has been shown to target heterologous proteins to the plasma membrane (Hancock, J., *et al., EMBO J.* 10:4033 (1991)). The RNAses of this invention have identical uses.

Please replace the two paragraphs at page 37, lines 6-29 with the following amended paragraphs:

A DNA sequence corresponding to amino acid residues 16-98 of ~~Onconase~~ ONCONASE® was cloned by PCR amplification of *Rana pipiens* genomic DNA and sequenced. The sequence, consisting of 252 bp of DNA encoding the ribonuclease was designated *Rana* clone 9. Total cellular RNA was isolated from either male or female *Rana pipiens* tissues using

RNA STAT-60 (TEL-TEST "B", Inc.) according to the manufacturer's protocol. Poly A+ containing mRNA was prepared using an Oligotex mRNA kit (Qiagen). Poly (A+) RNA was size fractionated on a 1% agarose gel containing 6% formaldehyde and blotted onto Nitran® nylon membranes (Schleicher & Schuell) in 10X SSC overnight. The membrane was rinsed in 2X SSC for 5 min, air dried and the RNA was cross linked to the membrane by exposure to UV light (Ultra-Lum) for 2 min. The RNA blot was hybridized at 42oC for 16-18 hours with a [^{32}P]-labeled DNA probe prepared from 30 ng of *Rana* clone 9 insert using the oligo labeling kit from Amersham. After hybridization, the RNA blot was washed twice in 1X SSC, 1% SDS for 20 min at 42oC. The blot was exposed to X-ray film for 4 days. The molecular size of mRNA was estimated using 0.24-9.5 kb RNA molecular weight markers (BRL).

Since ~~Onconase~~ ONCONASE® is isolated in large quantities from the oocytes of *Rana pipiens*, it was assumed that high levels of RNAse RNA would be present in the mRNA from oocytes. Surprisingly, mRNA reacting with *Rana* clone 9 was not detected in *Rana pipiens* oocyte, heart, lung and kidney tissues. The only mRNA signal detected with *Rana* clone 9 was a strongly hybridizing 3.6 kb RNA in mRNA isolated from *Rana pipiens* liver. As a protocol control, the same northern blot was probed with a [^{32}P]-labeled human actin cDNA. Actin mRNA was detectable in all of the tissues. In another northern analysis with four-fold more liver poly (A+) mRNA, a second weakly hybridizing mRNA of about 950 bp was detected.

Please replace the two paragraphs at page 38, line 24 bridgin to page 39, line 8 with the following amended paragraphs:

To determine if RNAse is present in *Rana pipiens* oocytes or other tissues, protein extracts were isolated from various *Rana pipiens* tissues and separated on a 4-20% Tris-Glycine SDS- containing polyacrylamide gel. The protein extracts were transferred to a nitrocellulose membrane using 1X transfer buffer (Novagen) at 250 mA for 45 min. The membrane was probed with primary and secondary antibodies as described in Chen, *et al., Oncogene* 12:241 (1996). The primary anti-ONCONASE® ~~Onconase ®~~ antibody was used at 1:100 dilution. The

detecting antibody (horseradish peroxidase labeled donkey anti-rabbit Ig (Amersham)) was used at 1:2500 dilution. The antibodies were visualized using an ECL detection kit from Amersham.

The western blot analysis demonstrated that a protein of the correct size (12 kDa) was present in extracts from oocytes. Other tissues, including liver, did not contain a 12 kDa protein that reacted with the anti-ONCONASE® ~~Onconase®~~ antibody. High molecular weight bands were also observed. These represented other forms of ~~Onconase~~ ONCONASE® (e.g., glycosylated or multimeric) or represented related members of the pancreatic ribonuclease A amphibian superfamily. It had been previously determined that the anti-ONCONASE® ~~Onconase®~~ antibody cross reacts with other pancreatic type RNAses such as bovine pancreatic ribonuclease as well as two human RNAses; eosinophil-derived neurotoxin and angiogenin.

Please replace the paragraph at page 39, line 30 bridging to page 40, line 7 with the following amended paragraph:

Clone 5a1b cDNA (SEQ ID NO:17) which was about 2.8 kb in size, contained an open reading frame (ORF) at the 5' end. The deduced amino acids at positions 1-23 were characteristic of a signal peptide with a charged amino acid within the first 5 amino acids, a stretch of at least 9 hydrophobic amino acids to span the membrane, and a cysteine at position 23. The putative signal peptide sequence was followed by a highly conserved but not identical amino acid sequence compared to ~~Onconase~~ ONCONASE®. There were four amino acid differences between the ORF of clone 5a1b and ~~Onconase~~ ONCONASE ® including amino acid residues 11, 20, 85 and 103. With the exception of a conservative change at amino acid residue 11, all the other amino acid conversions are between polar and charged amino acid residues.

Please replace the paragraph encompassing Table 1, lines 1-8 with the following amended paragraph:

Table 1. Ribonuclease activity

RNAse	RNAse Activity (units/mg protein)	Fold Increase
native ~~Onconase~~ ONCONASE®	9	
recombinanat *Rana Catesbeiana* RNAse	200	22
recombinant ~~Onconase~~ ONCONASE® (Q1S)	1.5	
recombinant *Rana pipiens* RNAse (Q1S)	2.5	1.7

Please replace the two paragraphs at page 44, lines 20-30 with the following amended paragraphs:

Recombinant ~~Onconase~~ ONCONASE® with a methionine at the -1 position was not very cytotoxic since correct hydrogen bonding at the active site is fostered by the pyroglutamic acid N-terminus of the native protein (Newton, *et al., Protein Engineering* 10:463 (1997)). In the four human tumor cell lines tested, the recombinant *Rana pipiens* liver RNAses were more active than recombinant ~~Onconase~~ ONCONASE®. It appears that the four amino acid differences in RaPLR1 change the active site configuration such that is does not display the degree of dependence ~~Onconase~~ ONCONASE® has on the N-terminal pyroglutamic acid residue for correct hydrogen bonding at the active site.

Similarly, RaCOR1 was also more cytotoxic than recombinant ~~Onconase~~ ONCONASE®. Again, most likely this is due to an active site that is not dependent on the N-terminal pyroglutamic acid for correct hydrogen bonding.

Please replace page 45 of the specification showing Table II with Replacement page 45 attached in Appendix A. A marked-up version showing the changes to Table II is included in Appendix A.